ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

REDACTORES:

E. MUSTAKALLIO (TURKU) U. UOTILA (HELSINKI) ARMAS VARTIAINEN (HELSINKI)

A. VILSKA (HELSINKI) A. I. VIRTANEN (HELSINKI)

EDITOR

K. O. RENKONEN

REDIGENDA CURAVIT

M. TUOMIOJA

THE EFFECT OF THYROID POWDER ON THE LETHAL DOSE OF ADRENALINE

BY

PENTTI PELTOLA

VOL. 28 1950 SUPPLEMENTUM 4

FROM THE UNIVERSITY INSTITUTE OF PHARMACOLOGY, HELSINKI
ACTING CHIEF: PROFESSOR A. VARTIAINEN, M.D.

THE EFFECT OF THYROID POWDER ON THE LETHAL DOSE OF ADRENALINE

by

PENTTI PELTOLA

HELSINKI 1950

Helsinki, 1950. Kirjapaino Oy. Liike

PREFACE

On the completion of the present part of my investigations into the determination of the biological activity of thyroid preparations, I wish to express my great gratitude to the acting Chief of the University Institute of Pharmacology, Professor Armas Vartiainen, M.D., for his suggesting the subject of the work, for the interest shown by him and for the valuable advice and instruction he has given me at the different stages of the work. I also wish to thank him for my introduction to pharmacological research. My debt to him for this guidance has grown with the years.

I also wish to record my gratitude to the former Chief of the Institute, Professor Yrjö Airila, M.D. (died 1949), who always contributed with a great deal of helpful advice to the progress of my work.

The statistical treatment of the results of my work was carried out by Mr. Tauno Jylhä, M.A., and Mr. Martti Kajamaa, M.A., to whom I wish to extend my best thanks.

Further I wish to thank Mrs. Hilkka Mäkinen, M.A., and Mr. L. A. Keyworth, M.A. (Cantab.), for the translation of my work into English, and Dr. Tauno Mustanoja, Ph.D., Docent, for its linguistic editing.

For the present research I received a State Subsidy for Junior Scientists in 1948.

Helsinki, April 27, 1950

Pentti Peltola

CONTENTS

INTRODUCTION

In the course of the last twenty years, along with the rapid development in other branches of medicine, great progress has been made in the field of hormone research. It has been possible to isolate almost every known hormone, and the chemical construction and even the synthetic production of the majority is well known. This results in the availability of chemically pure substances for the use of different hormones in therapy, the dosage of which can be accurately regulated according to weight, and methods of standardising hormone preparations, in most cases, have become obsolete.

The thyroid hormone, too, has been known for years. It was first isolated by Kendall in 1919, and its chemical structure was solved by Harington 1926. Similarly, its synthetic preparation has been known for years (Harington and Barger 1927). In therapy, however, the thyroid hormone or thyroxine has proved fairly troublesome, and uncertain of effect. Taken orally its effect is uncertain and varies greatly in individual cases. Subcutaneously injected its effect is of short duration, and a prolonged hormone treatment — which is usual with the use of the thyroid hormone, would be inconvenient from the patient's point of view. For this reason clinical practice still resorts, generally, to preparations containing dried animal thyroid powder. The biological effectivity of this powder has been determined by standardisation according to different methods, employing some standard preparation of known effectivity as the object of comparison. Such standardisation,naturally, must be effected separately for each lot produced, as the activity of thyroid powder varies considerably.

On the other hand, however, none of the standardising methods in use at present has proved accurate enough, and considerable differences exist between the different lots of production even when the same method is employed. The inaccuracy of the methods is perhaps best evidenced by their great number; within one country

even the different medicine manufacturers may employ different standardisation methods to determine the effectivity of their preparation. It is therefore natural that differences present between production lots where a single method is used multiply with the employment of several methods. *E.g.* the Scandinavian Standardisation Commission (Nordiska Specialitetskomissionen) (1932) has been able to establish multiple differences between the preparations of different medicine manufacturers. From the point of view of the treatment of the patient, the dissimilarity of the preparations is most confusing and lacking in reliability; accurate standardisation must be considered of great therapeutical importance and the creation of a reliable standardisation method of first-rate urgency. The importance of the question is perhaps well illustrated by the fact that a permanent Scandinavian Thyroid Standardisation Committee has been engaged on this task since 1946.

EARLIER STANDARDISATION METHODS

Of the dozens of methods of standardising thyroid preparations 3 or 4 only are in general use at present. These methods may be based on: *a.* changes occasioned by thyroidectomy, *b.* changes taking place in normal animals, and *c.* chemical methods. The methods most used at present are those based on changes in normal animals and the chemical ones.

METHODS BASED ON THE PREVENTION OF SYMPTOMS OCCASIONED BY THYROIDECTOMY

The methods of this group are based on the prevention, with the aid of thyroid preparations, of the retardation of growth and development due to thyroidectomy performed on growing animals. They are readily apparent in comparing thyroidectomised animals with normal ones, and the biological activity of the thyroid preparation in question can be judged by the size of the dose required to prevent the backwardness of growth and development of the animals.

The Method Based on the Retarded Growth of Thyroidectomised Goats and Sheep (Pick and Pineles 1910), also studied by Todd and Wharton (1934) The disadvantages of the method are its poor sensitivity, the high price of the test animals, and the slowness of the method, as the reaction must be observed for several months while the animals grow.

Method Based on Tests Carried out with Cretine Rabbits (Basinger 1916). — With this method, thyroidectomy is performed on 2—3-week old rabbits, and the thyroid preparation to be examined is administered to the animals either curatively, to cure symptoms caused, or prophylactically, to prevent their occurrence after thyroidectomy. The disadvantage of this method too is its slowness. The variation in the weight gained by the animals and in the eczemas

caused by hypothyroidism must be observed for a period of over 20 weeks at least, a considerable drawback in practice. In addition, the method has not proved very sensitive (Trendelenburg and Vogt 1937).

The Method Based on Observing the Weight Gained by Thyroidectomised Rats (Smith 1927), in which no distinct difference between normal and thyroidectomised animals can be registered, either, until after 1—2 months, and the method must therefore be considered very slow.

The methods in this group can be summed up as being very specific, and very suitable for qualitative tests. On the other hand, they show little sensitivity, *i.e.* to establish the changes occasioned by different doses the differences between the doses must be very great. Furthermore, the tests are very slow to carry out, taking several weeks and often months, which causes great difficulties in practical standardisation. Hence the methods of this group are not very generally employed in determining the effectivity of thyroid preparations.

METHODS BASED ON CHANGES IN NORMAL ANIMALS

The majority of the methods in use at present are based on the effect of thyroid powder on healthy animals. The biological reaction, on which these reactions are based, may consist of:

Loss of weight of test animal, or change in lethal dose:

a. the method based on the variation of the lethal dose with the guinea-pig (Freud and Nobel),
b. mouse weight method (Haffner and Komijama),
c. guinea-pig weight method according to Kreitmair,
d. Cameron's rat method.

Change in gas metabolism:

a. the method based on the variations in the amount of gas metabolism,
b. the method based on specific dynamic variation,
c. the method based on increased sensitivity towards oxygen deficiency.

Sensitivity to temperature after thyroid effect and procaine temperature reaction (Glaubach-Pick).

Reduction in the glycogen content of the liver.
Increased nitrogen secretion in urine.
Tendency of thyroid powder to increase the acetonitrile resistence.
Tendency of thyroid powder to stimulate metamorphosis:
a. in frogs,
b. in axolotls,
c. in salamander larvae.
Effect on feathers and pigmentation:
a. depigmentation method,
b. moulting method,
c. pigmentation method with re-growing feathers.

In addition, there is a number of methods generally considered to possess a limited efficiency, such as that based on increased excretion of water and salt, those based on the change in blood viscosity and protein content, change in nerve irritability, increased sensitivity to adrenaline in the blood pressure test, or in blood vein preparation according to Trendelenburg's frog test, and the methods based on changes in fat metabolism, on increased pulse rate, and on the complement content of blood.

METHODS BASED ON THE LETHAL THYROID POWDER DOSE OR REDUCTION IN THE WEIGHTS OF TEST ANIMALS

As is known, thyroid powder occasions considerable loss of weight in test animals, and the bigger the dose of thyroid powder the greater the loss. Long-term use of the powder or large single doses may even occasion the death of test animals. Both these phenomena have been employed as a basis for standardisation methods, *i.e.* loss of weight or death of the animals due to the powder. Each of the changes is based on the tendency of thyroid powder to stimulate metabolism, though this is hardly the only effective factor.

The common advantage of all the methods in the group is the technical ease with which the tests can be carried out. This group includes:

a. Method Based on the Lethal Dose for Guinea-pigs (Freud and Nobel 1924). — Thyroid powder is administered daily to the test animals with a sound, and the dose with which the animals die in approx. 10 days is determined. According to Rotter (1932) this

method of standardisation is appropriate as the results achieved by it are similar to those arrived at by Kreitmair's method and Harington's thyroxine determination method to be described below, and also correspond to some extent to the clinical results. The parallellism between iodine content and intensity of effect, however, cannot be definitively indicated (Solé 1928).

b. Mouse Weight Method According to Haffner and Komijama (1925). — In the same way as in the previous method, the preparation to be examined is administered to the animals, *i.e.* mice, by sound every morning for 8—10 days, and the animals are weighed regularly. Approx. 10 mg of dry thyroid powder given in the course of 8—14 days causes a 10 per cent loss of weight. The method must be considered a very approximative one, as 100 per cent differences in doses cannot be clearly indicated.

c. Guinea-pig Weight Method According to Kreitmair (1928). — This method is no longer employed for the standardisation of thyroid preparations. The guinea-pigs serving as test animals are given the preparation to be studied, also by sound, on six consecutive days. Reduction in weight sets in from the third day on, and continues for about a week in proportion to the size of the effective dose. The intensity of the effect is usually judged by the weight measured on the 7th day. According to Rotter (1932) the results obtained by this method agree with the results arrived at by the thyroxine determination method and also those achieved by Nobel's method. However, the method is hardly suitable for any but qualitative tests (Jorpes and Wilander 1933, Vartiainen 1950).

d. Cameron's Rat Method (1929) is based on the diminishing rate of weight increase and the hypertrophy of certain internal organs with growing rats that have been given thyroid powder over a lengthy period. The rats used as test animals are given the powder, on an average, for 6 to 9 weeks, after which their weight and the weight of certain internal organs, such as liver, kidneys, heart, adrenal glands and thyroid gland, is compared with the control animals. With those given thyroid powder the weight of the internal organs other than the thyroid gland exceeds that of the control animals, whereas the thyroid gland is smaller. The activity of the thyroid powder can be judged by the range of the differences. The method is slow, and not very sensitive.

The methods in this group can be summed up as being technically

fairly easy of execution, but due to their small degree of sensitivity they are unsuitable for standardisation purposes, except as qualitative tests.

METHODS BASED ON CHANGE IN GAS METABOLISM

In the methods of this group, the gas metabolism, accelerated by the intensified burning caused by thyroid powder, has been employed as the basis of reaction. The changes caused by thyroid powder in gas metabolism are very specific and fairly distinctly discernible. They are also considered so typical of thyroid powder that the use of at least one of the methods in this group as a standardisation method, in conjunction with any of the other methods that may be used, is generally regarded as necessary. The most usual methods in this group are based on:

a. Variations in the Amount of Gas Metabolism. — These variations can be proved in many different ways. The determination can take place by means of

— a closed respiration system, the determination being effected by analysing the air in the respiration chamber before and after the test, or by determining the consumption of oxygen by means of a spirometer (Benedict 1926, Krogh and Lindberg 1931, Hecht 1932 and Häusler 1936) or by means of a manometer (Foster and Sundström 1926, Krogh and Lindberg 1931, Jorpes and Wilander 1933, Aehle 1935 and Pelyi 1935) or by the Warburg principle (Büngeler 1930, Kanitz and Apitzsch 1934),

— open respiration systems according to the Haldane principle. This method involves the determination by weighing of the oxygen consumed and carbon dioxide exhaled by the test animal, the former *e.g.* by the increase in weight of the entire apparatus, and the latter bound with some substance binding carbon dioxide (Haffner 1927, Mörch 1928, Oberdisse 1931).

There are dozens of different apparatuses operating on the above principles to indicate gas metabolism, differing mutually in technical application for the most part.

A feature common to all the methods in this group can be said to consist of their requiring fairly complicated apparatuses and accurate control during the test. Furthermore, the test animals must be chosen carefully. *E.g.* small test animals mean that the

gas amounts in question are small, and that measuring errors and *e.g.* variations in temperature may cause considerable technical errors. Even such a slight change as a difference of 2° C in the temperature of test room may produce definitely disadvantageous effects. If the test is effected with large test animals, a natural consequence is a small number of tests, which reduces the accuracy of the results. In addition, it is difficult, or almost impossible, to exclude completely the change in gas metabolism occasioned by the work during the test. The primary consideration is the often very considerably accelerated gas metabolism occasioned by the movements of the test animal.

b. Method Based on the Variation of Specific Dynamic Effect. — The thyroid powder occasions a constant rise in the specific dynamic effect, even by as much as three to five times. A method serviceable for the determination of the range of this variation is to measure the consumption of oxygen and calculate the amount of heat it represents. The change in specific dynamic effect emerges after a few days from the administration of thyroid powder but is relatively unreliable for use as a standardisation method, as *e.g.* the specific dynamic effect of protein may be somewhat reduced, or slightly increased (Meyer 1929).

c. Methods Based on Increased Sensitivity to Oxygen Deficiency. — Due to accelerated basal metabolism occasioned by thyroid powder and the resulting increased consumption, the test animals that have been given thyroid powder are considerably more sensitive to oxygen deficiency than normal animals, which again are more sensitive than animals whose metabolic rate is below normal, *e.g.* through thyroidectomy. On this basis too methods have been developed for the determination of the biological activity of thyroid powder. In these tests the test animals are kept in an atmosphere of which the oxygen content is progressively reduced by the respiration of the test animal, and the animal's reactions in the different oxygen contents are observed (Asher and Streuli 1918, Takahashi 1924, de Quervain 1923, 1925, Marti 1923, Henning 1934).

A defect common also to these methods must be considered to consist of the increased burning due to movements of the test animals and the resulting accelerated consumption of oxygen, which again disturbingly affects the time within which the test animals die.

In addition, the methods are relatively little sensitive and require special apparatuses.

A method for which comparatively little special equipment is required is to seal the test animals in an air tight vessel and to measure the time they take to die. The disadvantage of this method also is the increased movement of the test animals due to their restlessness, and the convulsions commencing towards the end of the test particularly, both increasing the oxygen consumption considerably (Peltola).

METHODS BASED ON SENSITIVITY TO TEMPERATURE AFTER THYROID ACTION

Thyroid powder produces greatly increased sensitivity in test animals, *e.g.* rats, to variations in temperature. Normally, if the animal is placed in a temperature of $+28-30°$ C, there is no reaction, but after thyroid powder administration, intense perspiration and even dyspnea sets in. This method (Abelin 1923) is suitable primarily for qualitative tests only.

The group may be considered to include Glaubach-Pick's (1931, 1935) procaine temperature reaction. This refers to the fact that thyroid powder diminishes the rate of the temperature decline induced by procaine injection. The change may be very considerable. With normal animals it may extend to $+34°$ C even, but with animals to which thyroid powder has been administered it may not occur at all. The reaction to thyroid powder and thyroxine is specific, but not sensitive enough for quantitative determinations.

METHOD BASED ON THE REDUCED GLYCOGEN CONTENT OF THE LIVER

The method is based on the reduction in the glycogen content of the liver due to the effect of thyroid powder (Cramer 1913, Parhon 1913). The test animals are given thyroid powder over a period of some days (2 to 5), after which the animals are killed and the glycogen content of the liver determined both of normal animals and of those to which thyroid powder had been given. The glycogen content of the liver, which with normal rats, for instance, varies between 3 to 6 per cent, may, due to thyroid powder effect, be reduced in the course of the test to 0 per cent. The reaction is dependent on food. *E.g.*

an abundance of fat, and the feeding of the test animals with casein and a high amount of vitamins may entirely prevent any effect from the thyroid powder; this is the case with hunger, and against the influence of hunger thyroid powder is barely capable of reducing the glycogen content of the liver at all. This method has attained no general use in the standardisation of thyroid preparations as it is relatively difficult technically, and liable to the possible errors referred to above.

METHOD BASED ON THE EXCRETION OF NITROGEN IN URINE

Thyroid powder occasions acceleration of protein metabolism too, intensifying particularly the toxic decomposition of proteins and leading to increased excretion of nitrogen in the urine. The increased excretion of nitrogen, based almost exclusively on the excretion of urea and creatine nitrogen, is proportionate to the dose of the thyroid powder, but more specific still than the excretion of total nitrogen is the excretion of creatine and creatinine (Chick and Roscoe 1930). The execution of the tests is a fairly complicated matter, and a frequent source of error is the mingling of nutriments in the urine or stool discharged. The method is not generally used for standardisation.

METHOD BASED ON INCREASED ACETONITRILE RESISTANCE

In many test animals thyroid powder produces increased resistance against various poisonous substances, such as ether, paraldehyde, salvarsan, etc. A similar increase in resistance to acetonitrile is observed in mice too, whereas the case is the opposite with rats and guinea-pigs: they become more sensitive to acetonitrile. Administering of thyroid powder may very considerably increase the lethal dose of acetonitrile, even by 20 times, but does not affect the sensitivity to other nitriles. The Hunt (1905, 1910, 1923, 1925) method, based on this reaction, is at present a common standardising method for thyroid powder preparations. Thyroid powder is given to test animals either once only, according to Haffner (1925), or on three consecutive days, according to Grab (1932), or for 7—14 days, according to Hunt, after which the lethal dose of acetonitrile is determined. The reaction is technically easy to occasion, but it has the drawback of several great potential errors. A nutritional change alone, from food

containing carbohydrates to that of protein content, occasions the reduction of the lethal dose of acetonitrile to 1/30 (Hunt) of the ordinary lethal dose. Similarly, the vitamin content of food plays an important part. A-vitamin, in particular, reduces the resistance considerably (Hunt). In addition, the resistance is very greatly dependent on the temperature of the surroundings, light (Santo 1934) and season of the year (Grab 1932).

However, the acetonitrile method is one of the standardisation methods employed often at present, and the reliability of the method may increase fairly considerably if the technique employed on each occasion is as much the same as possible. A considerable part, naturally, is played by the degree of experience of the person carrying out the test. The question of the specificity of the acetonitrile reaction has also been much discussed. It has been found that the blood of thyroidectomised cats is similar in effect to thyroid powder (Trendelenburg 1910, Miura 1922). Similarly, a thyroidectomised mouse is more resistant against acetonitrile than a normal one (Paal 1933). Both of these observations counter fairly strongly the specificity of the reaction. Several investigators, in fact, take a negative view of the method, *e.g.* Gellhorn (1923), Goldner (1928), Kreitmair (1930), basing their assertions *e.g.* on the fact that great differences exist between the different mouse races. Gaddum (1931) too adopts a negative attitude to the reaction, as he has been able to find but a fairly slight similarity between the effect of the examined substances and their chemical content of thyroxine. Haffner's modification, in particular, according to which thyroid powder is administered on one day only, has been strongly opposed, as the effect of such a dose differs considerably from the change in basal metabolism occasioned by a similar dose.

On the other hand, a close similarity exists between the increase of acetonitrile resistance due to thyroid preparations, and the iodine content. The test results have become more accurate also now that the detrimental factors are known. Carried out according to the present methods, the reaction is one of the methods used for standardisation of thyroid preparations today.

METHODS BASED ON THYROID POWDER ACCELERATING THE METAMORPHOSIS

It is known of old that the thyroid gland greatly accelerates metamorphosis of the larvae of certain cold-blooded animals (Gudernatsch 1913). If the larvae of frogs *(Rana, Bufo* or *Bombinator)* are kept in thyroxine solution or fed with thyroid powder, their metamorphosis is so considerably accelerated that the metamorphosis, which with a certain frog species *(Rana catesbiana)* normally takes 2 to 3 years, under the influence of the powder takes place in three weeks. A similar change takes place also in salamander larvae and even in axolotl larvae, which latter, in normal conditions, never undergo a metamorphosis. With frog larvae, the thyroid powder, even in a solution of 1 : 1 000 000, may still produce a definite acceleration of the metamorphosis.

Frog Larva Method. — The test animals employed are the larvae of either *Rana esculenta, Rana temporaria* or *Bufo vulgaris.* Most suitable are the larvae of *Rana temporaria,* those of *Rana esculenta* being more susceptible to by-effects. The larvae of *Bufo vulgaris,* again, are less sensitive in the test itself. In the course of the test the animals are kept in white or yellowish vessels, in which the water is changed every second day, and the temperature kept as constant as possible, approx. +20° C. The slightest changes in temperature may produce considerable errors; *e.g.* at +30° C the effect of the thyroid gland ceases, and at approx. + 7° C the metamorphosis is completely discontinued (Belkin 1934). The thyroid powder is administered to the larvae either by adding it to the water or mixing it with the food. The metamorphosis is observed by photographing the larvae at certain intervals and observing the development of the different parts of the body. The method is not absolutely specific. *E.g.* certain amino acids also tend to accelerate the metamorphosis (Gudernatsch 1933), as does ergosterine (Romeis 1913). On the other hand, some amino acids, such as arginine and ornithine (Gudernatsch 1933), and also quinine (Belkin 1934) tend to offset the thyroid gland effect. The reaction is dependent on other factors as well, such as the acidity of the water in which the larvae are kept, its calcium and potassium content, phosphates, ferrous salts, and other chemical factors. Furthermore, the method is easily serviceable in the Spring only. It is generally reckoned that this method indicates 100—200 per cent differences between doses.

Axolotl Method. — The animals employed in the tests are 6 to 8 months old, kept, separately, during the test, in an aquarium at constant temperature, and they receive the substance to be examined in a single administration by sound into their stomachs, or intraperitoneally in tablets. The metamorphosis produced by thyroid powder is slower with axolotl than with frog larvae, taking about 4 weeks. The metamorphosis of the animals is best observed photographically, and the change is judged primarily by the size of the branchiae and the formation of the tail sail (Abelin 1923). The method is suitable to some degree for quantitative studies, and suffices to indicate approximately 100 per cent differences in doses. The axolotl method is still being actively studied, and is obviously also being developed; to some extent it is likely to be used also for practical standardisation purposes.

Salamander Larva Method. — The test animals used are salamander larvae, best collected as salamander eggs and raised in aquaria up to a stage enabling them to be used for the tests. The substance to be examined is perhaps best administered to the animals together with the worms intended for their feeding, and the observing of the metamorphosis takes place in the same way as was described above for frog and axolotl larvae. The reaction of the animals is greatly dependent on many different factors, *e.g.* temperature, light, nutrition, acidity of water (Uhlenhuth 1922, 1929), etc. The problem of acquisition of test animals, their feeding with special nutrition, etc., have resulted in the fact that the reaction has not attained any great popularity as a method of standardisation.

The standardisation methods based on thyroid powder accelerating the metamorphosis have proved impractical in operation, being slow, involving the difficulties of obtaining the test animals and feeding them, and being also fairly little sensitive, and are therefore not very widely used.

EFFECT OF THYROID POWDER ON FEATHERS AND PIGMENTATION

In birds the thyroid powder causes, apart from moulting, the growth of feathers, but with a simultaneous change of feather type. Female animals react more readily than the males. Large quantities of hormones also occasion the disappearance of pigmentation from

the feathers while small doses add a certain pigment, particularly to breast and back feathers. Attempts have been made to base standardisation methods on these reactions too.

Depigmentation Method. — Any dosage of thyroid powder causes a retarded pigmentation, disturbing the formation of red pigment particularly, which is replaced by the more resistant black. Large doses of thyroid powder occasion the general disappearance of colour from the feathers. The test animals usually employed in the method based on this reaction are hens, from which a part of the feathers is removed primarily from the neck, close to the median line. With normal birds, the new feather growth is exactly similar to the old, while the colour is definitely different in those treated with thyroid powder. Certain conclusions on the activity of the thyroid powder can be drawn from the size of the dose occasioning a change in colour (Zawadowsky 1932). The method is impractical, relatively inaccurate and takes time; one test can be estimated to take at least two months.

Moulting Method. — In the moulting method also a similar dependence on sex is apparent as in connection with depigmentation (Zawadowsky 1932), and it is completely, or in the majority of cases at least almost completely, inhibited by the influence of sex hormones. It is therefore dependent on the condition of the animal's own sexual glands. The method is not, either, suitable for quantitative determinations.

Pigmentation Method on Re-growing Feathers. — The method is based on the augmentation of black colour, due to thyroid powder, in the re-growing feathers (Juhn and Barnes 1931). The animals used for these tests are light-brown hens from which feathers are removed from certain places about three weeks before the test starts. Due to thyroid powder, augmentation of colour down to the base of the feathers is seen in the re-growing feathers. This reaction becomes visible a few days after administration of the thyroid powder, and may begin after a single dose. Depending on the size of the dose, the pigmentation is present on areas of varying extent, and conclusions as to the activity of the thyroid powder can be drawn from the size of these patches. The reaction is specific. The method is suitable, primarily, for qualitative tests only, while it is uncertain of result in quantitative tests.

The standardisation methods in this group can be considered suitable for very rough quantitative examinations but best for qualitative tests, for which the last-mentioned method in particular, giving a specific and quick reaction, is very suitable indeed.

OTHER METHODS

In addition to the above-mentioned, there is a great number of standardisation methods based on a great variety of reactions, but they have proved more or less unreliable or unspecific and have not been accepted for general use. Among them may be mentioned the methods based on the determination of blood viscosity and protein content, excretion of salts and water, changes in fat metabolism, increased pulse rate, changed complement content of blood, nerve irritability, and sensitivity to adrenaline.

Method Based on the Determination of Blood Viscosity and Protein Content. — As early as in 1910 it could be shown that thyroidectomy occasioned an increased blood viscosity (Gardella 1910, Paladino 1912, Deusch 1922). Attempts have been made to utilise this as a standardisation method, and *e.g.* Abelin and Sato (1925), in animal tests, have found corresponding changes after the administration of thyroid powder. However, the method has not proved quantitatively suitable.

Method Based on Excretion of Salts and Water. — Eppinger showed (1917) that the excretion of salts and water increase after the administration of thyroid powder. An attempt was made to utilise this observation for standardisation *e.g.* with a dog to which 500 cu.cm. of 2 per cent saline solution was administered orally. In normal conditions, approx. 23 per cent of the salt, and 1/5 of the quantity of liquid consumed is excreted in three hours. After administration of thyroid powder the excretion of salt may increase to 43 per cent and the excretion of urine may be doubled. It is possible that the influence of hormone on the protein is a factor concerned here. This method has not, either, proved suitable for quantitative tests.

Method Based on Changes in Fat Metabolism. — Thyroid hormone fairly rapidly, in 1 to 3 days, causes a considerable reduction in the fat content of the muscles in particular, which may even amount to 70 per cent. Loss takes place also in the fat content of the liver, up to 30 per cent. Attempts have also been made to employ this reaction

as a standardisation method with rats, treated for a few days with the preparation to be tested, after which the fat content of liver and muscles was compared with the corresponding figures for the normal animals (Abelin 1928). This method has not become very popular either.

Method Based on Increased Pulse Rate. — Thyroid powder, as is known, causes tachycardia, and attempts have been made to utilise this in standardisation, primarily with dogs and also with rats (Smith 1927). However, the method is dependent on several error factors, *e.g.* movements and state of irritation of test animals, for which reason it is not suitable for quantitative tests.

Method Based on the Complement Content of Blood. — Thyroid powder produces a reduced complement content of blood. However, this is not proportionate to the reduction in the glycogen content of the liver, which would indicate a non-specific effect. The same effect is also produced by cholesterine and certain substances of antithyroid effect (Fischer and Loew 1934), which effect favours more strongly still non-specificity.

Method Based on Nerve Irritability and Sensitivity to Adrenaline. — The thyroid powder produces increased nerve irritability, seen *e.g.* from the fact that under the influence of thyroid powder the animal reacts to milder electric irritation than an animal in normal conditions. Attempts have been made to utilise this as a standardisation method by irritating the depressor nerve of a rabbit in narcosis (Asher and Streuli 1918), or the vagus nerve of a cat or a dog, or the splanchnic nerve (Fürth and Schwarz 1908).

Another attempt has been made to adapt sensitivity to adrenaline for standardisation, *e.g.* by studying the sensitivity to adrenaline produced by thyroid powder by a blood pressure test (Kraus 1908, Asher and v. Rodt 1912, Oswald 1916), or by the Trendelenburg frog test, in which the reaction to adrenaline of a vein isolated from the general blood circulation is studied (Asher 1911, Eiger 1917, Csillag 1924). These reactions, however, could not be considered as specific to thyroid powder, but it has been possible to show that *e.g.* serum and Witte's peptone, protein, plasma or amino acids produce similarly increased sensitivity to adrenaline (Storm van Leuwen and van den Made 1920, Freund and Gottlieb 1922, Abderhalden and Gellhorn 1923, Csillag 1924), and therefore these methods too have not been accepted for general use.

CHEMICAL METHODS

Attempts have also been made to solve the difficult problem of the determination of the biological activity of thyroid powder by a purely chemical course. In this respect the standardisation has generally aimed at the chemical quantitative determination, either of the iodine content of the preparations, primarily of the organically bound iodine, or of their thyroxine content. The prerequisite for a successful standardisation of this kind is that the quantitatively determined factor and the biological activity are parallel completely.

In studying organically bound iodine, it has been possible to ascertain that a part of it, 5—50 per cent, is conjugated to thyroxine (Kendall 1928, Leland and Foster 1932, Harington 1933, Blau 1935), and the balance, 50 per cent or more, in some other form, primarily as diiodotyrosine, which represents over 30 per cent of total iodine (Harington and Randall 1929, Foster 1929) and which is much less effective than thyroxine, possibly even, in part, its counter-acting agent (Abelin and Wegelin 1932). As, therefore, the quantities of thyroxine and diiodotyrosine, both in their absolute values and mutual relationship, may vary considerably — which also applies to their biological activity — the determination of total iodine cannot be considered as indicative of the biological activity (Kocher 1917, Fukui 1925, Engländer 1925).

Also, the determination of thyroxine content is an unreliable basis for a standardisation method. For instance, thyroid powder has a much stronger effect than the corresponding amount of iodine in the form of thyroxine. Similarly, if thyroid powder is given continuously a reduction in its activity is noticeable, which is not the case with thyroxine (Abelin 1928). Further, the activation of thyroid powder by the anterior lobe hormone of the pituitary gland may increase the gas metabolism more rapidly and intensely than even a 100-fold dose of thyroxine (Reiss 1932). Therefore, the thyroxine determination cannot, either, be considered as suitable for the determination of the activity of thyroid powder.

Of the chemical methods the one most likely to give an accurate result is perhaps the determination of the total iodine, which coincides most with the biological activity (Gaddum 1930, Salter and Lerman 1935), but it still seems evident that in determining the activity of thyroid powder a biological test must be considered indispensable.

The chemical method may only support the value determination arrived at in the biological way.

Hence, at least for the time being, the biological standardisation method must be considered the most reliable indicator of the activity of thyroid powder.

CONCLUSIONS

The literature dealing with the standardisation of thyroid preparations can be summarised as follows:

Dozens of different methods have been developed for the standardisation of thyroid preparations, all based on the extremely varied reactions which may be produced by thyroidectomy or by the use of thyroid preparations.

Most of these methods are very inaccurate, *i.e.* error margins are very great, and they are therefore suitable primarily for qualitative tests only. A number of them, again, are but little specific, and several substances other than thyroid preparations may produce a similar reaction, for which reason these methods are less suitable as standardisation methods. In addition, many of them are difficult to carry out in practice, and technical and other secondary factors may cause considerable disturbances in judging the test results.

None of the methods mentioned, singly, has proved adequate for the standardisation of thyroid preparations. Two or several must be used parallelly to attain sufficient accuracy.

As is obvious from the above, numerous different methods have been developed to determine the biological activity of thyroid preparations, of which none has proved sufficiently reliable or advantageous. As the standardisation of thyroid preparations has a primarily practical end, the author finds that a satisfactory method should fulfil at least the following conditions:

The method should be sensitive enough, *i.e.* it must be able to show such variations in the strength of the different preparations as may produce a differing effect on the patient. The majority of the methods mentioned above can only indicate a 100 per cent or greater difference between doses, and but a few show differences below 100 per cent. Most sensitive among them are probably the acetonitrile reaction and the method based on basal metabolism, with which approximately 30 per cent differences between the various doses can be traced.

The method should be as little dependent as possible on external factors. In this respect too the methods described above are far from ideal. For instance, in acetonitrile reaction a mere change from a carbohydrate diet to protein food may cause even thirty times greater differences in the size of the lethal dose of acetonitrile alone (Hunt 1925); similarly, the movements of test animals in the basal metabolism test may result in quite great errors.

The method should be so easy of experimental execution that the effect of the technical errors dependent on the personal factor are reduced to a minimum and the test is made as simple as possible to carry out. The ideal obviously, would be a method based on a reaction in which the possibility of misreadings is reduced to a minimum. The reaction primarily entering into question would be *e.g.* that causing the death of the test animal.

As the thyroid preparations to be standardised are intended for clinical use, the method to be sought after for standardisation purposes should be one based on some animal phenomenon as closely related as possible to the reaction produced by thyroid preparations in man. In such a case it could be expected that the intensity of the reaction, both in animal and man, would be best comparable mutually by the dose.

In addition, the endeavour should be to choose a test animal as closely related as possible to man even as regards the digestion, preferably at least from among the higher mammals so that the changes produced by thyroid preparations should bear as close a mutual similarity as possible. It must also be borne in mind that organic activities depending on, *e.g.* the regulation of temperature and other vegetative functions, may be very differently organised in the different species — the temperature regulation system is entirely absent in *e.g.* poikilothermal animals — while many observations demonstrate that thyroid activity and vegetative functions are very closely correlated mutually. It is also important that the test animals should be easily available, easy to keep and inexpensive, to enable their abundant use and thus increase the accuracy of a quantitative experiment.

It would also benefit the reaction if the test could be effected in as short a space of time as possible, since the problem is to find a method to serve practical requirements. Long test periods considerably increase research expenses and make adequate repeat

tests difficult. This is typical *e.g.* of tests carried out on strumectomised animals.

The above considerations seem to the author of importance in studying standardisation methods of thyroid preparations, and his endeavour has been to follow them in investigating reactions that might prove suitable as the basis of a standardisation method.

OBJECT OF THE PRESENT INVESTIGATION

In studying, in the course of several years, the effect of thyroxine and other thyroid preparations on basal metabolism, and also the effect of certain substances, affecting primarily the vegetative nerve system, on thyroxine-accelerated basal metabolism, the author has on several occasions ascertained the close connection between hyperthyreosis on the one hand and simultaneous sympathicotonia on the other (Peltola and Vartiainen 1945). It has been possible to find by several different methods, including clinical, that considerably increased sensitivity to adrenaline is present in thyrotoxicosis. This can be found *e.g.* by administering 1 : 1 000 adrenaline solution drops into the eye. If the person in question is in a state of thyrotoxicosis the pupil will expand considerably, whereas no expansion occurs in the pupil of a normal person (Goetsch 1922). Similarly, surgeons have been able to show several times that in operations on toxic goitre, during or after the application of local anesthetics, the patient may suddenly die for no particular external reason, and it has been found that this occurs when the local anesthetic contains adrenaline (Paus 1947). The conclusion drawn from this phenomenon is that death in these cases has been due to greatly increased sensitivity to adrenaline in thyrotoxicosis (Wahlberg 1938); the injection of an equal amount of procaine into a patient in a state of thyrotoxicosis generally produces no complications. There are several other observations distinctly indicative of greatly increased sensitivity to adrenaline in thyrotoxicosis. As, in the present writer's opinion, the phenomenon is very marked, typical of thyrotoxicosis and distinctly observable in animals also, it was considered justifiable to investigate its quantitative dependence on the degree of thyrotoxicosis and on other factors possibly affecting the reactions with a view to its suitability as a standardisation method for thyroid preparations.

It is true that attempts have been made to apply the increased sensitivity to adrenaline produced by thyroid preparations as a

standardisation method *e.g.* with the aid of the blood pressure test (Kraus, Asher, Oswald) and by studying, in Trendelenburg's frog test, the sensitivity to adrenaline of a blood vessel preparation under the influence of thyroid preparations (Asher, Eiger, Csillag). However, both of these methods have been given up because of the inaccuracy and non-specificity of test results. As, however, no mention has been found in literature of other tests on these lines, and as the potential errors in the above-mentioned tests, in the form of both test errors and errors caused by external factors, are considerable, it was considered necessary to investigate the possibility of developing a method which would be as little dependent on test errors as possible. In this way a reaction was arrived at based on the variation in the lethal dose of adrenaline, due to thyroxine, in the test animals — and as far as is known this method has not been studied previously. In the following investigation the endeavour has been to throw light on the factors affecting the size of the lethal dose of adrenaline in normal animals and in animals to which thyroid powder has been administered, as well as the quantitative proportions between the different factors. The object of the investigation can be summarised in the following points:

Is the lethal dose of adrenaline constant with the different animals, or is there any variation? If there is, what are the factors responsible for it and how extensive are the changes they cause?

Does the thyroid powder occasion a change in the lethal dose of adrenaline in the test animals and, if so, are the changes quantitatively dependent on the amount of thyroid powder administered to the animals and is the change possibly dependent on other factors and to what extent?

Is it possible to develop, on the basis of this reaction, a method for the determination of the biological activity of the different thyroid preparations?

MATERIAL AND METHODS

According to the requirements made of the method on p. 23, mice have been selected as test animals since, because of their small size and low cost, they can be used in fairly great numbers but, being mammals, are nevertheless as close to man as possible. The animals were albinos, and, as it was impossible to obtain such a large number from a single supplier, supplied mainly by three breeders in as large batches as possible in order to achieve the maximum uniformity of the animal material. The weight of the animals has varied, generally, between 20—30 g, a small percentage being under 20 g or over 30 g in weight. Ninety-five per cent of the animals were males. Females have been employed only to ascertain possible differences due to sex. The animals not employed have been kept in large batches to ensure as uniform conditions as possible.

In the course of the test each of the animals lived in a separate 2-litre vessel, and they were fed on white bread and water, in adequate quantities; the animals not employed received oats and milk in addition. The food given in the course of the test proved sufficient, and not even its long-term use produced any variation in the size of the lethal dose of adrenaline.

The thyroid preparation used was dried thyroid powder supplied by the Orion Pharmaceutical Manufacturing Co., originally imported from the U.S.A. According to the total iodine determination effected in the U.S.A. it contained 0.27 per cent iodine, and according to an analysis by Orion Co. 0.23 per cent. The supply used in the test was taken from one and the same carefully mixed lot and, stored in a glass vessel sealed with a paraffin-waxed cork, kept in a refrigerator at +4° C. The thyroid powder was administered to the animals in a water suspension of 2 1/2—5 per cent, freshly prepared each time and injected into a small quantity of white bread, care being taken to ensure that no suspension remained outside the bread. The piece

of bread was given to the mice in the morning after carefully removing the uneaten food from the previous day together with the water container in the vessel, thus preventing any food containing thyroid powder entering the water container. Usually the test animals finished the food containing thyroid powder within one or two hours, but they received no additional food or any water until the afternoon, when it was also checked that the piece containing thyroid powder had been eaten up.

The adrenaline used in the tests was the »Adrenal» preparation supplied by the Orion Co. and, unless otherwise stated, it was administered to the animals in a 1 : 1 000 concentration. The injections were effected subcutaneously, using a thin injection needle in order to keep the puncture in the skin as small as possible and prevent the adrenaline seeping out. Generally, the death of test animals occurred in the course of a few hours after the injection, but in reviewing the test results all the animals that died within 24 hours after the injection have been entered as deaths.

During the tests the animals were kept in a fairly well lit room, at a temperature varying between +16— +18° C.

In judging the test results the dose causing 50 per cent mortality (LD 50) was employed. In each of the tests it was obtained in the following way:

The test animals were injected with at least 5 different doses of adrenaline, the difference between the doses being 1 γ/g, calculated according to the weight of the animals, and each dose was injected into a minimum of 5 animals. When the LD 50 dosage failed to coincide with the mean of the test group the doses were increased or decreased until a minimum of two of the doses were greater and less than the LD 50 dosage. Hence, at least 25 animals were employed in each test to determine one LD 50, but in the majority of the cases considerably more, and the size of the dose causing LD 50 was confirmed by at least 2 consecutive doses of the next larger and 2 of the next smaller size.

TEST RESULTS

LETHAL DOSE OF ADRENALINE

A total of 374 animals was used to determine the lethal dose of adrenaline in tests carried out on normal test animals. The following are some of the conclusions which can be drawn from the test results, which are best seen from Table 1:

TABLE 1

LETHAL DOSE OF ADRENALINE. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 374 ANIMALS

Dose of Adrenaline γ/g	Mortality	Per cent
25	10/10	100
20	10/10	100
10	10/10	100
9	16/19	84
8	25/33	76
7	27/40	68
6	76/163	47
5	13/32	41
4	4/28	14
3	0/5	0
2	0/24	0

In injecting adrenaline in increasing doses of 2—25 γ/g, the mortality percentage of the test animals was found to be directly proportionate to the amount of adrenaline injected. The minimum dose lethal to test animals was 4 γ/g. Fifty per cent mortality was attained with 7 γ/g and 100 per cent with 10 γ/g. With the dose

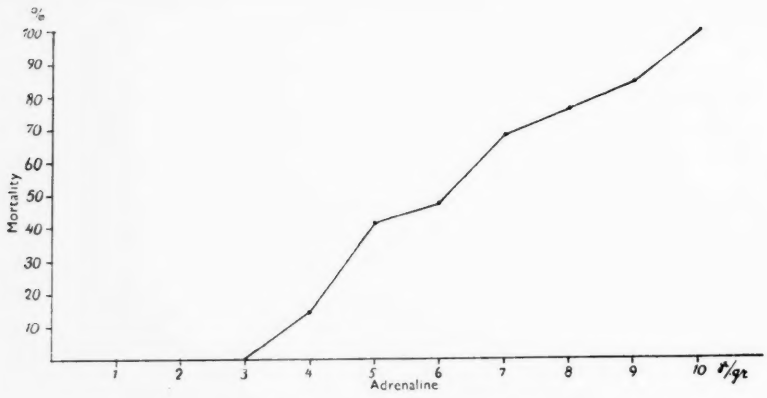

Fig. 1. — Lethal dose of adrenaline. Total 374 animals.

varying between 4—10 γ/g the increase in mortality percentage was a function of the adrenaline dose, as can be seen from Fig. 1.

Factors Affecting the Lethal Dose of Adrenaline. — Numerous tests have been carried out to throw light on the factors affecting the size of the lethal dose of adrenaline, the endeavour being to study its possible dependence on the factors which may most frequently produce errors in standardisation methods, such as the season of the year, lighting, weight of animals, sex, food, effect of fasting and also the concentration of the adrenaline injected.

Effect of the Season. — In studying the influence of several substances on the animals it has been possible to ascertain that it is greatly dependent on the season in which the test is carried out. For instance, it was found that the lethal dose of acetonitrile may vary very considerably, being about one-third in September of the lethal dose ascertained for December (Grab 1932). This naturally considerably hinders the standardisation to be effected at different times of the year. In investigating the effect of the season on the size of the lethal dose of adrenaline the animal material employed has been divided into two groups, one of them comprising the tests effected in the summer months and the other those effected in the winter months. Summer months are taken to be May 1 to October 1, and winter months October 1 to May 1, and a comparison has been carried out with each dose of adrenaline in each of the groups. The test results are best shown by Fig. 2; a study of this graph will reveal

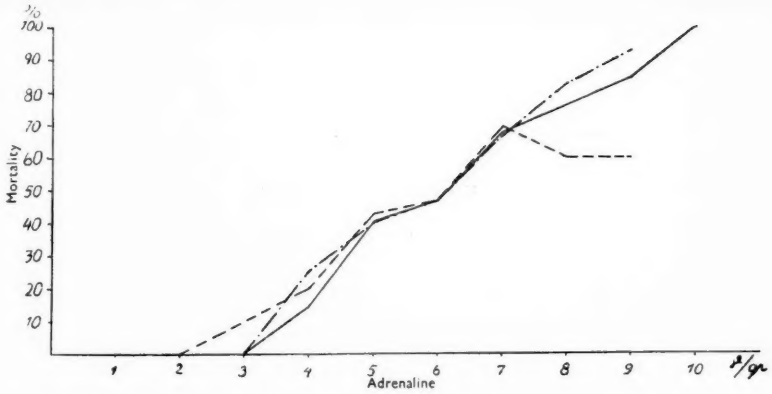

Fig. 2. — Dependence of the lethal dose of adrenaline on the season. Total 374 animals.
· — · — Winter, October 1 to May 1, 210 animals.
— — — Summer, May 1 to October 1, 164 animals.
——— Normal.

that no radical difference in mortality percentages exists between summer and winter months. At any rate it can be said that the possible changes are practically insignificant, no change being observed *e.g.* in the L D 50, which is 7 γ/g for each group.

Effect of Light. — It has long been known that the activity of the thyroid gland in well-lit surroundings is considerably less than in the dark, and it has even been possible to ascertain that darkness has caused pronounced hyperthyreosis in test animals (Loeser and Eitel 1932). Similarly, light has been found to produce changes *e.g.* in the size of the lethal dose of acetonitrile (Paal 1933, Santo 1934). From hyperthyreosis produced by darkness it could be assumed that the lethal dose of adrenaline also changes with test animals kept in darkness, and this in fact is the case, as is shown by Table 2 and Fig. 4. LD 50, which for animals kept in normal conditions is 7 γ/g, drops to 4 γ/g due to darkness. Similarly, a 100 per cent mortality is attained even with 8 γ/g, whereas normally it enters only with 10 γ/g. It seems therefore obvious that darkness fairly distinctly increases the animals' sensitivity to adrenaline.

Effect of the Weight of Animals. — As a variation in the physiological properties of animals used for standardisation may induce errors in the standardisation procedure, tests were also carried out with animals of different weights. The animal material employed is divided into four groups: under 20 g, 21—25 g, 26—30 g, and over 31 g.

TABLE 2

LETHAL DOSE OF ADRENALINE FOR TEST ANIMALS KEPT IN DARKNESS. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 45 ANIMALS

Dose of Adrenaline γ/g	Mortality
9	6/6
8	6/6
7	5/6
6	5/6
5	4/6
4	3/5
3	1/5
2	0/5

TABLE 3

EFFECT OF THE WEIGHT OF TEST ANIMALS ON THE LETHAL DOSE OF ADRENALINE. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 268 ANIMALS. SEE PAGE 46

Dose of Adrenaline D(a) γ/g	Weight g	Mortality (q)	q	1−q	$(q)=\sqrt{\frac{q(1-q)}{n}}$
8	31−	—	—	—	—
	26−30	9/9	0.67	0.33	0.16
	21−25	9/12	0.75	0.25	0.12
	−20	10/12	0.83	0.17	0.11
7	31−	0/1	0.00	1.0	—
	26−30	7/8	0.88	0.12	0.12
	21−25	12/18	0.67	0.33	0.11
	−20	8/13	0.62	0.38	0.14
6	31−	3/6	0.50	0.50	0.20
	26−30	14/35	0.40	0.60	0.08
	21−25	32/65	0.49	0.51	0.06
	−20	27/57	0.47	0,53	0.07
5	31−	1/1	1.00	0.00	—
	26−30	3/7	0.43	0.57	0.19
	21−25	7/20	0.35	0.65	0.11
	−20	2/4	0.50	0.50	0.25

TABLE 4

LETHAL DOSE OF ADRENALINE FOR FEMALES. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 66 ANIMALS

Dose of Adrenaline γ/g	Mortality
9	5/6
8	6/10
7	6/12
6	10/23
5	2/5
4	1/5
3	0/5

Comparisons have been carried out between the different weight groups with different doses of adrenaline. The results are given in Table 3. On the basis of the test results it seems that no regularity can be observed between the different groups when different sized doses were employed. It seems obvious, therefore, that the weight of the test animals does not affect the size of the lethal dose of adrenaline, at least not if adult mice are employed.

Effect of Sex. — To complement the tests regarding the differences in the animal material, investigations were also made into the effect of sex on the size of the lethal dose. Adult, non-pregnant females were employed for these tests. As shown by Table 4 and Fig. 4, LD 50 is the same for females as for males. No difference between the sexes is therefore discernible. No investigation was made into the possible changes occasioned by pregnancy as this was considered a question quite different from the purpose of the investigation.

Effect of Food. — In studying the potential errors in the different standardisation methods it has been found that variation in diet may occasion even large changes in the effect of thyroxine and thyroid preparations. Marked variations in the properties of the animals have been found even independently of thyroid effect, merely as a result of dietary variations. In acetonitrile reaction, for example, it has been found that the lethal dose of acetonitrile may differ merely as a result of changed diet, even by 30 times. In this case the lethal dose of acetonitrile is highest with animals fed on

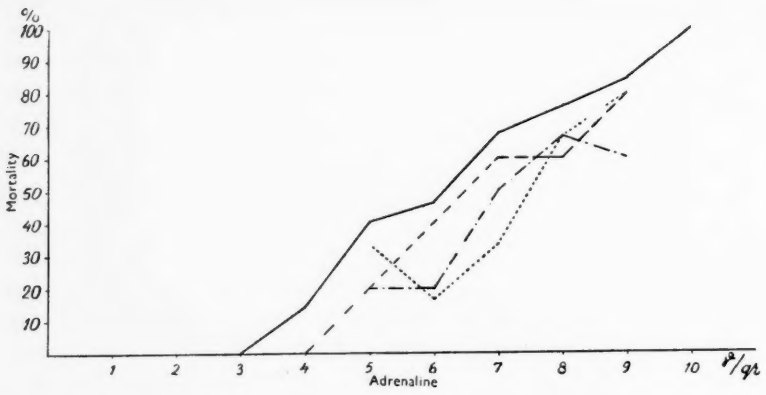

Fig. 3. — Effect of nutrition on the lethal dose of adrenaline. Total 86 animals.
— — — Group I: with ordinary test food, 25 animals.
· — · — » II: » milk, eggs, biscuits, 32 animals.
- - - - - - » III: » rolled oats, liver, 29 animals.
——— Normal.

oatmeal and liver, and lowest with those fed on milk, eggs and biscuits (Hunt 1925).

Investigations were also made into the effect of food on the lethal dose of adrenaline. The animals were divided into three groups: Group I, fed on white bread and water, *i.e.* the ordinary food used in the tests, Group II on milk, egg and biscuits, and Group III on rolled oats, liver and water. The test results are given in Fig. 3.

A study of the results shows that LD 50 among the animals of Group I, fed on the diet employed in the tests, is 7 γ/g, of Group II 7 γ/g, and of Group III 8 γ/g. Hence, it seems that LD 50 does vary slightly between the different nutritional groups, but food cannot be considered to have any great effect on the size of the lethal dose of adrenaline.

Investigations were made, in addition, into the possible interrelation of a short interval between the taking of the meal and the moment of injection. To this end the mice were made to fast for 24 hours. During this time they were given water at will. The test results are best seen from Fig. 4.

According to the test results it seems that LD 50 of adrenaline, after 24 hours fasting, is 5 γ/g, while for normal animals it is 7 γ/g, and consequently a 24-hour fast can perhaps be considered

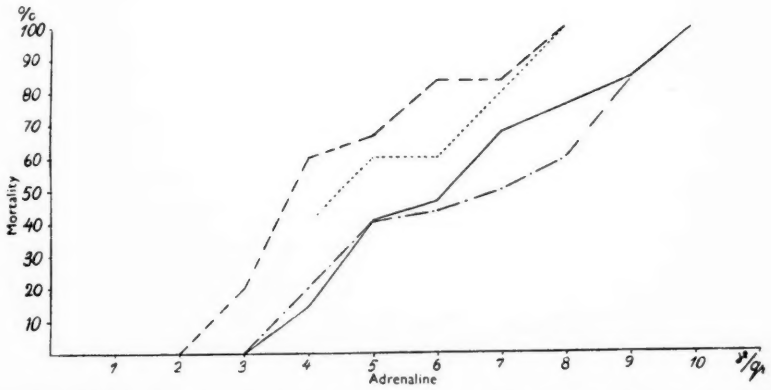

Fig. 4. —Effect of darkness, sex and fasting on the lethal dose of adrenaline. Total 146 animals.
— — — Group I: kept in darkness for fourteen days, 45 animals.
· — · — » II: females in normal conditions, 66 animals.
- - - - - - » III: after a fasting of 24 hours, 35 animals.
——— Normal.

to reduce to some extent the size of the lethal dose of adrenaline, probably the result of a deterioration in the general condition from fasting.

Effect of Concentration of the Adrenaline Injected. — The intensity of the effect of several subcutaneously injected substances is independent of the concentration in which the substance is injected under the skin, provided the variations in concentration are not considerable. Adrenaline, however, tends to contract the blood vessels considerably, which results in retarded resorption of the solution containing adrenaline in the area of injection. This has been utilised *e.g.* to extend the influence of local anaesthetics by adding to them small quantities of adrenaline.

No mention has been found in the literature as to the extent to which the tendency of adrenaline to retard the resorption affects the activity of adrenaline itself in the different concentrations. For this reason it was considered necessary to investigate the effect of adrenaline concentration on the size of the lethal dose. Adrenaline was injected in the tests in the concentrations 1 : 1 000, 1 : 2 000, 1 : 3 000, 1 : 4 000 and 1 : 5 000. The test results are seen from Fig. 5.

A study of the test results shows that LD 50, 7 γ/g with a 1 : 1 000

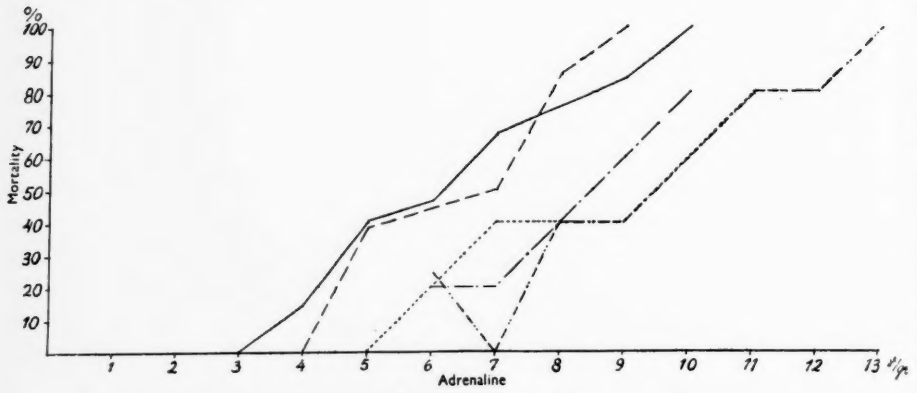

Fig. 5. — Effect of adrenaline concentration on the lethal dose of adrenaline. Total 149 animals.

— — — Group I: adrenaline concentration 1 : 2000, 49 animals.
· — · — » II: » » 1 : 3000, 25 animals.
- - - - - - » III: » » 1 : 4000, 35 animals.
·· — ·· — » IV: » » 1 : 5000, 40 animals.
— Normal.

solution, is also 7 γ/g with a 1 : 2 000 solution. With greater dilutions LD 50 begins to rise, and is 9 γ/g in a 1 : 3 000 concentration, 10 γ/g in 1 : 4 000 as well as in 1 : 5 000. From the results it seems obvious that the effect of adrenaline in concentrations of 1 : 1 000—1 : 2 000 is not dependent on the concentration of the adrenaline solution, but that in greater dilutions LD 50 increases considerably.

Effect of Temperature. — Similarly to light, temperature also has been found in many different tests to be closely connected with the activity of the thyroid gland. With an animal kept in a cold place a distinct change indicative of increased activity is found in the thyroid gland within about 5—7 days, particularly in the microscopic structure of the gland (Kuschinsky 1935). Low temperature also occasions an increase in the lethal dose of acetonitrile (Hunt 1923) and, with frog larvae, it accelerates the metamorphosis caused by thyroxine (Woitkewitsch 1935). In the basal metabolism test also changes in temperature may evoke considerable differences (Mörch 1928).

It could naturally be expected that the lethal dose of adrenaline is also reduced with animals kept at a low temperature to accord with accelerated activity of the thyroid gland, and, hence, is increased with animals kept at higher temperatures.

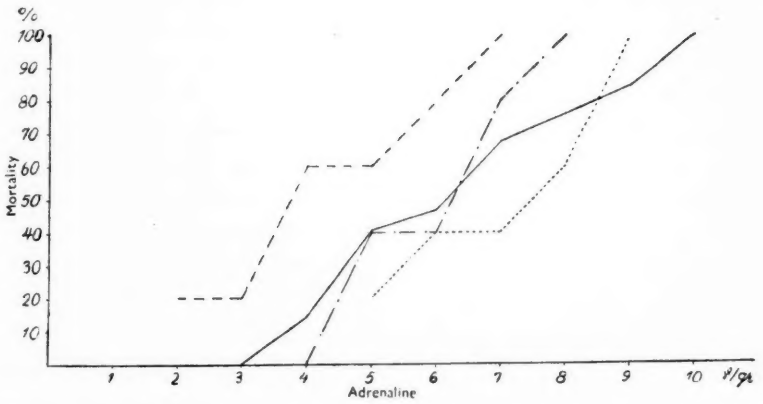

Fig. 6. — Effect of temperature on the lethal dose of adrenaline. Total animals 95.
— — — Group I: kept at 10° C, 35 animals.
· — · — » II: kept at 18° C, 30 animals.
- - - - - - » III: kept at 30° C, 30 animals.
——— Normal.

To study this question, test animals were kept at three different temperatures, +10° C, +18° C and +30° C. The test results are best seen from Fig. 6.

On the basis of the test results it seems that the LD 50 of adrenaline with test animals kept at a low temperature (+ 10° C) is 4 γ/g, at + 18° C 7 γ/g and at + 30° C 8γ/g. It must therefore be considered obvious that cold occasions a fairly distinct reduction in LD 50, while heat tends to increase it, which phenomena evidently must be connected with the respectively increased or reduced activity of the thyroid gland.

SUMMARY

The investigations into the lethal dose of adrenaline (more correctly, LD 50) and into the nature of the factors affecting it, can be summarised as follows, on the basis of the tests described above:

The LD 50 of adrenaline in a 1 : 1 000 concentration is approx. 7 γ/g with a mouse injected subcutaneously. The LD 50 varies to some extent with the different mouse strains but not greatly, generally keeping within the limit 1 γ/g (page 47), provided the animals

are kept in completely similar conditions. Some difference may occur due to the effect of the food, but these variations are fairly small. On the variation of light and temperature the lethal dose of adrenaline shows greater dependence; a rise in temperature occasions an increase in LD 50, while keeping the animals in darkness reduces the LD 50 down to 4 γ/g. Further, the LD 50 is dependent on the concentration of the adrenaline solution, provided the differences in dilutions are great, *e.g.* an increase in the dilution from 1 : 1 000 to 1 : 3 000 occasions a rise of LD 50 from 7 γ/g to 9 γ/g. Smaller variations, 1 : 1 000—1 : 2 000, cause no distinct change in LD 50. On the other hand, the effect of adrenaline appears to have no relation to the weight of the test animals, insofar as adult mice are concerned, nor to sex, provided the test animals are not pregnant, nor any distinct relation to the season of the year. A 24-hour fast occasions a reduction in LD 50, but this may be due to a deterioration in general condition.

EFFECT OF THYROID POWDER ON THE LD 50 OF ADRENALINE

In carrying out qualitative tests regarding the effect of thyroid powder on the lethal dose of adrenaline it was found with a few test animals that thyroid powder occasions a drop in the LD 50 of adrenaline and that the drop is dependent on the period during which the powder is administered to the test animal. For this reason it was considered necessary first to carry out tests to ascertain the period within which the thyroid powder, to produce a marked effect, should be administered to the test animals, and also to find out to what extent the effect is dependent on the time of administration.

Effect of the Time of Administering Thyroid Powder. — The tests were carried out with two different doses of thyroid powder, viz. 5 mg/20 g and 9 mg/20 g, of which the former produces a medium strong and the latter a strong reaction, on a total of 348 test animals. The tests were executed as described on page 27, the dose of thyroid powder being administered daily in a water suspension injected into a small amount of bread, and the adrenaline injections being made at intervals of 4 days. The test results are given in Fig. 7.

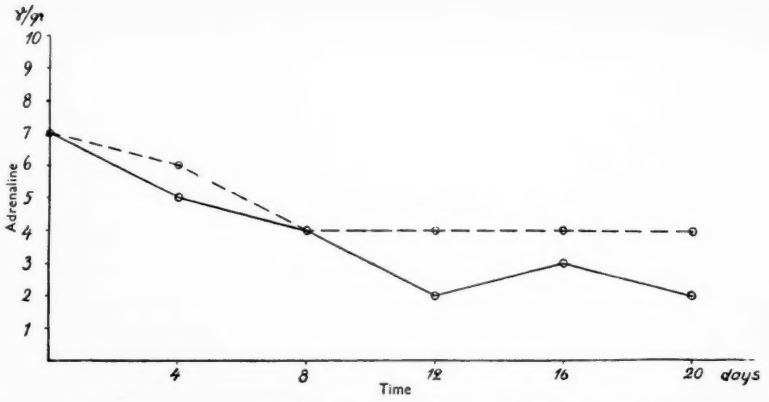

Fig. 7. — Effect of the time of administering thyroid powder on LD 50 of adrenaline. Total 348 animals.
——— 9 mg/20 g thyroid powder, 150 animals.
— — — 5 mg/20 g » » 198 animals.

The following conclusions can be drawn on the basis of these results:

With the 5 mg/20 g dose the LD 50 declines, from its initial value of 7 γ/g, to 6 γ/g in four days, 4 γ/g in 8 days, at which level it then remains even after 20 days, independent of whether the administering of the powder is continued or not. With the larger dose (9 mg/20 g) a more marked effect is produced; after 8 days the LD 50 drops to 4 γ/g and after 12 days to 2 γ/g, at which level it then stays.

On the basis of the test results it seems that thyroid powder occasions a considerable reduction in the lethal dose of adrenaline, and the reduction is dependent on the period of thyroid powder administration. The effect is at its strongest when the animals have been given thyroid powder for a minimum of 12 days. The effect is visible, although less marked, after 4 days, and quite distinctly apparent after 8 days. It attains its maximum only after 12 days and remains the same irrespective of continued administering of the powder at least up to the 20th day. This phenomenon seems the same even with different sizes of thyroid powder doses.

On the basis of the above, thyroid powder has been given to the animals for 12 days in the following tests, unless otherwise stated.

Effect of the Thyroid Powder Dose on the Lethal Dose of Adrenaline. — In the test to ascertain th quantitative interdependence of the

lethal dose of adrenaline and the amount of thyroid powder, the total number of animals employed was 256. They were given thyroid powder in increasing doses, 1—30 mg/20 g, in the course of 12 days. Of the animals to which larger doses, 30 mg/20 g, were given, a large part died during the test, obviously due to the effect of thyroid powder. It has already been found to produce the death of a mouse in a daily dose of 30—50 mg/20 g (Hesse, Jacobi and Bregulla 1933.) The death of the test animals in these cases took place 8—12 days after the thyroid powder dosage. The test results are best seen from Fig. 8.

In studying the test results the following observations were made:

The LD 50 of adrenaline is very considerably reduced by thyroid powder, from 7 γ/g to 1 γ/g even. The decline follows linearly the increase in the thyroid powder dosage, as long as the dose is 1 to 9 mg/20 g, and LD 50 is 2 γ/g if the test animals have been given 9 mg/20 g of thyroid powder. Subsequently, LD 50 declines very little, and for instance with 15 mg/20 g it is 1 γ/g, remaining about the same irrespective of increases in thyroid powder dosage. The same phenomenon is observed in studying other physiological changes produced by thyroid powder. *e. g.* in following the basal metabolism in mice accelerated by thyroid powder. The acceleration of basal metabolism, to a certain limit, is dependent on the amount of thyroid powder, but after that limit increased dosage no longer

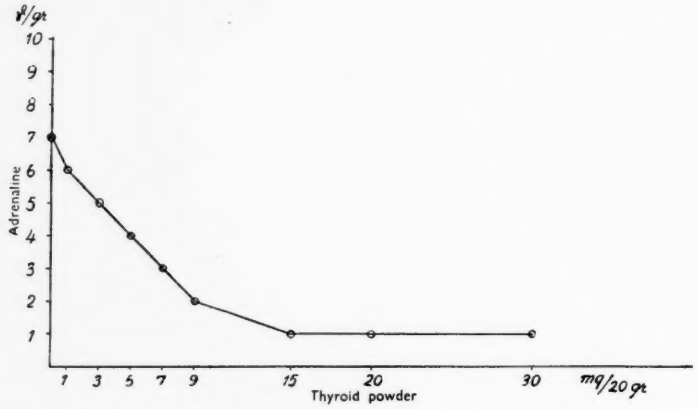

Fig. 8. — Dependence of LD 50 of adrenaline on the dose of thyroid powder. Total 256 animals. Mean error 1 γ/g.

accelerates the basal metabolism. Further, it can be concluded from the test result that while the thyroid powder dose is kept within 1—9 mg/20 g, the LD 50 with each dose differs so much that the deviation exceeds the variations occasioned by possible test errors. With larger doses the changes are less marked.

Dependence on Different Factors of the Change Produced by Thyroid Powder in the Lethal Dose of Adrenaline. — In studying the effect of thyroid powder it has been possible to prove by several different tests that its activity is dependent on quite a number of subsidiary factors which may have a considerable influence in determining its effect. Thus, in the acetonitrile test for instance, distinct seasonal variations have been observed in the changes occasioned by thyroid powder in the lethal dose of acetonitrile (Paal 1933, Santo 1934). Similarly, it has been possible to ascertain that the change occasioned by thyroid powder in basal metabolism is dependent on the temperature of the environment (Mörch 1928). It was also considered necessary, with regard to the change in the lethal dose of adrenaline occasioned by thyroid powder, to investigate the dependence of the reaction on certain subsidiary factors. Investigations were made into the effect of the season, lighting, nutrition, sex and temperature. In all these tests the dose of thyroid powder employed was the same, 5 mg/20 g, producing a medium strong reaction, and the animals were given it for 12 days.

Effect of Season. — Seasonal effect on the activity of the thyroid gland has been known for years; *e.g.* the thyroid gland activity of crows has been found to vary even by 8 times according to the season of the year (Küchler 1935). Similarly, it has been possible to ascertain that thyroid powder or thyroxine given to rabbits has a considerably more accelerating effect on metabolism in winter than in summer. For this reason it was considered necessary to investigate whether the reduction in the lethal dose of adrenaline, due to thyroid powder, would also be dependent on the season in which the test was carried out. For this purpose the tests were divided into two groups: those carried out in the winter months, from October 1 to May 1, and those in the summer months, May 1 to October 1, and a comparison was effected between the groups of results obtained in this way.

The test results are given in Table 5.

TABLE 5

EFFECT OF SEASON ON THE VARIATION IN LD 50 OF ADRENALINE OCCASIONED BY THYROID POWDER. SUMMER: MAY 1 TO OCTOBER 1. WINTER: OCTOBER 1 TO MAY 1. DOSE OF THYROID POWDER 5 Mg/20 g FOR 12 DAYS. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 81 ANIMALS

Dose of Adrenaline γ/g	Mortality in Summer	Mortality in Winter
7	7/8	7/8
6	6/8	7/8
5	5/8	5/8
4	4/8	3/5
3	2/5	2/5
2	1/5	0/5

On the basis of the test results it seems that the change occasioned by thyroid powder in the lethal dose of adrenaline is the same in the different seasons. The results obtained are similar in tests carried out in summer and in winter. In both cases, with 5 mg/20 g of thyroid powder, the LD 50 declines from 7 γ/g to 4 γ/g.

Effect of Light. — Variations in lighting, too, have been found to produce changes in the intensity of the effect of thyroid powder, *e.g.* in the acetonitrile reaction, as mentioned on p. 15. In the tests carried out it has been possible to show that the variations in the lethal dose of adrenaline are more dependent on the effect of light than is the variation of the lethal dose of adrenaline without the effect of thyroid powder. Test results are given in Table 6.

Effect of Sex. — In tests with males and females to throw light on the possible variations due to sex in the lethal dose of adrenaline, additional tests were included to ascertain the interdependence of the effect of thyroid powder in this respect as well. Forty-five males and 35 females were employed in the tests. The results are given in Table 7.

A study of the results shows that no difference can be observed in the size of LD 50 of adrenaline between male and female mice. It can therefore be considered as evident that the sex does not affect the extent of the change occasioned by thyroid powder in the lethal dose of adrenaline.

TABLE 6

EFFECT OF LIGHT ON THE LD 50 OF ADRENALINE OCCASIONED BY THYROID POWDER. DOSE OF THYROID POWDER 5 Mg/20 g FOR 12 DAYS. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 84 ANIMALS

Dose of Adrenaline γ/g	Mortality in Light	Mortality in Darkness
9	6/6	—
8	6/6	—
7	5/6	6/6
6	5/6	6/6
5	4/6	5/6
4	3/5	4/6
3	1/5	3/5
2	0/5	2/5
1	—	1/5

TABLE 7

EFFECT OF SEX ON THE LD 50 OF ADRENALINE OCCASIONED BY THYROID POWDER. DOSE OF THYROID POWDER 5 Mg/20 g FOR 12 DAYS. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 80 ANIMALS

Dose of Adrenaline γ/g	Mortality of Males	Mortality of Females
9	6/6	—
8	6/6	5/5
7	5/6	5/5
6	5/6	4/5
5	4/6	3/5
4	3/5	3/5
3	1/5	2/5
2	0/5	0/5

Effect of Food. — In order to throw light on the significance of food as a producer of possible changes in this respect, tests were carried out in the way described on p. 34 in regard to the lethal dose of adrenaline. Test results are given in Table 8.

TABLE 8

EFFECT OF NUTRITION ON THE LD 50 OF ADRENALINE OCCASIONED BY THYROID POWDER. DOSE OF THYROID POWDER 5 Mg/20 g FOR 12 DAYS. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 93 ANIMALS.
I WITH ORDINARY TEST FOOD
II WITH MILK, EGGS, BISCUITS
III WITH ROLLED OATS, LIVER

Dose of Adrenaline γ/g	Mortality Group I	Mortality Group II	Mortality Group III
7	5/6	5/5	5/5
6	5/6	4/5	5/5
5	4/6	3/5	3/5
4	3/5	2/5	2/5
3	1/5	1/5	0/5
2	0/5	0/5	0/5

As a summary of the test results it can be said that thyroid powder administered in doses of 5 mg/20 g occasions an equal reduction in LD 50 in the three different groups of animals: Group I received food normally used in these tests, Group II food containing milk, eggs and biscuits, and Group III liver, rolled oats and water. The change, therefore, does not seem to depend on the quality of the food received by the test animals.

Effect of Temperature. — To study the variations occasioned by temperature tests were carried out with a total of 80 test animals. A part of these animals had been kept for a fortnight at a temperature of +30° C, after which they also were given 5 mg/20 g of thyroid powder for 12 days. Test results are given in Table 9.

In studying the test results it can be found that the reduction in the lethal dose of adrenaline occasioned by thyroid powder was more marked with test animals kept at +30° C than with those kept at ordinary temperatures. The limits of the change were 8 γ/g and 4 γ/g. With animals kept at a lower temperature, again, the change is smaller in extent, evidently due to the decline in the lethal dose of adrenaline due to the cold alone. It therefore seems that the change occasioned by thyroid powder varies to some extent with test animals kept at different temperatures, and temperature thus does affect the reduction occasioned by thyroid powder in the lethal dose of adrenaline.

TABLE 9

EFFECT OF THYROID POWDER ON LD 50 OF ADRENALINE WITH TEST ANIMALS KEPT IN TEMPERATURES OF 18° C AND 30° C. DOSE OF THYROID POWDER 5 Mg/20 g FOR 12 DAYS. NUMERATOR-NUMBER OF DEATHS. DENOMINATOR-TOTAL NUMBER OF MICE. TOTAL 80 ANIMALS

Dose of Adrenaline γ/g	Mortality at 18° C	Mortality at 30° C
9	6/6	—
8	6/6	5/5
7	5/6	5/5
6	5/6	4/5
5	4/6	4/5
4	3/5	3/5
3	1/5	1/5
2	0/5	0/5

SUMMARY

The tests described above may be summarised as follows:

Thyroid powder, if administered to the test animals on several consecutive days, occasions a marked reduction in the lethal dose of adrenaline. The reduction is seen regularly, even in repeated tests, and its extent is proportionate to the amount of thyroid powder per weight unit. The effect sets in, in a less marked form, within 4 to 8 days if the thyroid powder has been administered daily, but maximal reduction is only attained after about 12 days, after which it remains approximately the same, irrespective of the continued administering of thyroid powder. The reduction occasioned by thyroid powder in the lethal dose of adrenaline, on the basis of the tests carried out, seems to be independent of the season of the year, sex of the test animals and their nutrition. On the other hand, light and temperature affect the change to some extent, but this effect, at least in part, is due to the change in the lethal dose of adrenaline occasioned by these factors.

STATISTICAL TREATMENT OF MATERIAL

In studying the effect of adrenaline on test animals the size of the adrenaline dose has been expressed in γ, so that 1 γ is equal to 0.001 mg of adrenaline per gram weight of the test animal. The adrenaline dose expressed in γ is denoted by D(a). The value of adrenaline dose with which the relative mortality of test animals is 50 per cent has been determined by the tests. The size of this dose, denoted by D (50 %, a) has been determined by carrying out the test with several consecutive total doses, and the value of D (50 %, a) represents the minimum value of the dose in γ with which the relative mortality is at least 50 per cent. The first part of the investigation deals with the possible dependence of adrenaline effect on the conditions in which the test animals were kept, whether this effect is different with different sexes, etc.

The second part of the investigation studies how the effect of adrenaline on test animals changes when the animals, previous to the administering of adrenaline, are given thyroid powder in standard daily doses. The value of D (50 %, a) arrived at is denoted by D (50 %, a, t). Apart from its determination in normal conditions, the value of D (50 %, a, t) has also been determined in other conditions, as has its dependence on the size, time of administering, etc., of the thyroid powder dose.

To investigate the method of observation itself, a study was made first of whether the keeping of the size of γ directly proportionate to the weight of the test animal could be considered appropriate. The test was carried out by dividing the test animals into classes according to weight, and the relative number of deaths was determined separately in each weight class with several values of adrenaline dose D(a), and the dispersion of the ratio obtained.

The following symbols are used:

$q = q\ (D)$ = relative mortality with adrenaline dose D

$q = \frac{v}{n}$ = value of q obtained by the test

n = number of test animals

v = number of deaths

$\sigma(q)$ = dispersion of relative mortality

It is assumed here, as generally in this investigation, that relative mortality follows the binomial distribution law. Hence,

$$\sigma(q) = \sqrt{\frac{q(1-q)}{n}}$$

From the results obtained in this way it was found that the relative mortality, within the limits of observation accuracy, is independent of the weight of test animals. The test results are given on page 32.

In studying the accuracy with which the values of D (50 %, a) can be determined from the tests carried out, it must be stated first that, when the value of D (50 %, a) is represented by the lowest value of D giving a relative mortality of at least 50 %, the error in rounding off will average 0.5 γ. Further, the value of D (50 %, a), as the tests show, depends on the prevailing conditions. No actual tests were carried out to determine this source of error, but, for instance, obviously due to this source of error is the systematic difference between the values of q(D) obtained in normal conditions and those obtained with normal feeding while the effect of feeding was being studied. See Fig. 3 on page 34.

Finally, the accuracy of the result obtained is dependent on the number of tests carried out, *i.e.* the number of test animals. As above,

$$\sigma(q) = \sqrt{\frac{q(1-q)}{n}}$$

Assuming that $q \simeq 0.50$ we obtain

with $n = 5$ $\quad \sigma(q) = 0.22$

» $n = 10$ $\quad \sigma(q) = 0.16$

» $n = 25$ $\quad \sigma(q) = 0.10$

On the basis of the results obtained in the various tests, a variation of 0.20 in q corresponds to an approximate change of 1 γ in the adrenaline dose D(a). The number of observations, n, being 5, $\sigma(q)$ correponds to an approximate variation of 1 γ in the adrenaline dose; with $n = 25$ $\sigma(q)$ is approx. 0.5 γ.

On the basis of this consideration of errors it can be concluded, although the probable range of the error caused by a possible change in conditions is very uncertain, that the dispersion of the values of D (50 %, a) or D (50 %, a, t) can sufficiently accurately be assumed to be independent of the number of observations, and from the test results it has been estimated that

$$\sigma\ (\mathrm{D}\ (50\ \%,\ \mathrm{a})\ ) \simeq 1\ \gamma$$
$$\sigma\ (\mathrm{D}\ (50\ \%\ \mathrm{a},\ \mathrm{t})\ ) \simeq 1\ \gamma$$

Differences smaller than the above even though systematical, may be due to the method used, and no reliable conclusions can be based on them.

After the values of D (50 %, a) were determined in two different cases, the question had subsequently been solved of whether the effect of adrenaline in these two cases was significantly different or whether the difference was due to a mere coincidence. The procedure used was as follows:

The symbols were

q_0 = value of relative mortality in normal case,
q_1 = value of relative mortality in cases differing from the normal.

It was first ascertained that the difference between the relative mortality values $q_0(D)$ and $q_1(D)$ was sufficiently great to obviate the error due to the method. Hence, the rounding-off error present in the determination of the D (50 %, a) value can be deleted from this error.

$$q_0(D) = \frac{v_0(D)}{n_0(D)}\ ;\ q_1(D) = \frac{v_1(D)}{n_1(D)}$$

On the basis of these the weighted mean value has been calculated:

$$\bar{q}_0(D) = \frac{v_0(D) + v_1(D)}{n_0(D) + n_1(D)}$$

In the cases in which the result of study indicated that the effect of adrenaline is essentially different, q(D), with all D values, has generally always been either smaller or greater than the corresponding $q_0(D)$.

If, in the tests,

$$q_1(D) < q_0(D),$$

the probability has been determined:

$$P\left(\frac{v(D)}{n_1(D)} \leqq \frac{v_1(D)}{n_1(D)}\right) = P(D)$$

P(D) indicates the probability, provided there is no significant difference between the test series when, with adrenaline dose D the test was carried out with n_1 test animals out of which v_1 died, that the number of deaths would have been v_1 animals or less.

According to binomial distribution:

$$P(D) = \sum_{r=0}^{v_1} \binom{n_1}{r} \bar{q}_0(D)^r \left(1 - q_0(D)\right)^{n_1 - r}$$

If, in the tests,

$$q_1(D) > q_0(D),$$

the corresponding formula employed has been:

$$P(D) = \sum_{r=v_1}^{n_1} \binom{n_1}{r} \bar{q}_0(D)^r \left(1 - \bar{q}_0(D)\right)^{n_1 - r}$$

By multiplying together the values of P(D) obtained with different D values, and supposing there is no significant difference between the test series, we will obtain the probability that by repeating test series No. 1 we would obtain the values for $q_1(D)$ that differ at least as much from the values of $\bar{q}_0(D)$ as the values actually obtained.

We denote

$$P_0 = P(D_1) \cdot P(D_2) \cdots P(D_m)$$

If $0.01 < P_0 \leq 0.05$ the difference is considered almost significant,

if $0.001 < P_0 \leq 0.01$ the difference is considered significant,

if $P_0 \leq 0.001$ the difference is considered highly significant.

If the distribution law is normal, the probability is that the observed value of the quantity differs from its probable value by

more than the amount of dispersion ..... approx. 0.33
more than twice the amount of dispersion ..... » 0.05
more than three times the amount of dispersion » 0.003

If the division is not normal these probabilities are generally higher than the above values.

DISCUSSION

As pointed out in the introduction, there is no current method of standardising thyroid powder that is accurate enough, easy of technical execution, to the greatest possible extent independent of external factors, and cheap. Of the methods in use at present, *e.g.* acetonitrile reaction, axolotl reaction, the reaction based on the metabolism of mice, and certain others were described in the first part of this paper, together with their most important disadvantages.

The endeavour of the present investigation is to develop a new reaction, one not so far investigated, to serve as a basis for standardisation, viz. the reducing effect of thyroid powder on the lethal dose of adrenaline. The increased sensitivity to adrenaline produced by thyroid powder has been known for years from both experimental and clinical research, but quantitative research into the effect of thyroid powder on the lethal dose of adrenaline is not known to have been carried out earlier.

As the sensitivity to adrenaline from increased thyroid gland activity is increased in human beings also, and as it proceeds on parallel lines among animals, particularly mammals, the author finds that it offers a suitable basis for standardisation. The reaction in question in the different animals is similar to that in man, and consequently the results obtained from test animals are best coordinated with the reaction produced by thyroid preparations in human beings, which is the reason for the standardisation. In this respect the method differs very considerably from the acetonitrile reaction for example, where acetonitrile tolerance has been found to increase in mice while it decreases with rats. Axolotl reaction again is based on the accelerated metamorphosis of a larva of the poikilothermal axolotl, a phenomenon that has no parallel produced by thyroid powder in man. The dissimilarity of the reaction, therefore, might prove a considerable drawback in the comparison of the effect of the thyroid preparation on axolotl and man.

The methods based on increased gas metabolism are, it is true, built on a reaction similar in animals and men. The common disadvantage inherent in the methods of determining gas metabolism, however, can be considered the fact that, in carrying out the measurements, errors occasioned by the movements of the test animals are very difficult, and almost impossible, to obviate. The tests require, in addition, large and complicated apparatus, for which reason they cannot be considered as suitable for ordinary standardisation.

The methods based on variations in weight of the test animals also depend on a reaction similar in animals and men, but show little sensitiveness, primarily due to the variation in the weight of the test animals, also normally present.

The above sources of error have been excluded in this work, and therefore the reduction in the lethal dose of adrenaline occasioned by thyroid powder must be considered very suitable for standardisation, as far as method is concerned.

In studying the test results attention has been paid first to the lethal dose of adrenaline and the factors that might occasion variations in it. The lethal dose of adrenaline, in stabilised conditions, seems to remain very constant, the LD 50 being 7 γ/g. The increase in mortality percentage with the different doses is remarkably sharp, which enables fairly distinct definition of the LD 50.

Variations in LD 50, according to investigations made, may be occasioned to some extent by the dissimilarity of the mouse strains; however, variation between the different strains does not usually exceed 1 γ/g. Between animals of the same strain variations are hardly noticeable.

Variations may also apparently originate in the temperature at which test animals are kept. The variations may extend up to 3 γ/g. Similar variations are involved in the majority of other methods also, *e.g.* in the acetonitrile reaction, and also in the method based on gas metabolism. This is natural as low temperature occasions a hyperfunction of the thyroid gland in test animals, which is then cumulative with the effect of the thyroid preparation to be standardised, causing an apparently larger reduction than usual. Increase in temperature, again, reduces the activity of the thyroid gland in the test animals, and in such a case the change observed in the test results is mainly due to the preparation to be standardised,

and therefore less in extent. However, variations in temperature must be considerable in order to occasion marked changes in the present method. This means that among animals kept at ordinary room temperature (16—18° C) the change in question can be disregarded.

The effect of light, too, is to some extent similar to that of temperature. Darkness causes a reduction in the lethal dose of adrenaline, making the test animals more sensitive to adrenaline, its effect therefore being the same as that of thyroid powder. This phenomenon can be explained in the same way as that produced by cold: in darkness, the activity of the animals' own thyroid gland is accelerated, resulting in a state corresponding to hyperthyreosis, occasioning a similar reaction to that caused by the administration of thyroid powder to the animals. This source of error, too, is easily excluded by keeping the test animals in even light. The same potential error is also present, *e.g.*, in acetonitrile reaction and the method based on basal metabolism.

On the other hand, the lethal dose of adrenaline seems to be independent of the food on which the test animals are fed. In this the method is considerably more favourable than the asetonitrile method, where a dietary change may, as mentioned before, occasion even 30-fold variations in the size of the lethal dose of acetonitrile.

In addition, the reaction is independent of the sex of the test animals employed, provided they are not pregnant, a point that considerably facilitates the availability of test animals.

Nor can any seasonal variation be shown in the lethal dose of adrenaline, at least not in tests carried out in summer and winter. This seems natural in fact, as the animals employed for the tests came from stocks that had lived in laboratory conditions for tens of generations and had consequently grown throughout the year in the same conditions, the effect of seasons naturally being slight. Furthermore, the size of the lethal dose of adrenaline is independent of the weight of the test animals, provided the animals are adults and heavier than 15 g. This too greatly facilitates the selection of test animals. On the other hand, the concentration of the adrenaline used for injections seems to occasion quite considerable variation in the size of the lethal dose of adrenaline, insofar as the differences in dilution concerned are large. This must be considered as natural since adrenaline in great dilutions has been dissolved in a considerable

quantity of liquid, which of course retards resorption. In these great dilutions too, however, the concentration is so high that the blood vessel contraction produced by adrenaline is maximal. The smallest differences, between 1 : 1 000—1 : 2 000, on the other hand, occasion no distinct change in the lethal dose of adrenaline. The size of the lethal dose of adrenaline, on the basis of the investigations reported above, seems to be fairly constant, if the test animals are kept in similar conditions. The factors that may produce changes in this respect are very easy of exclusion in laboratory conditions, and similarly, of course, the potential errors occasioned by them are easily eliminated.

A study of the effect of thyroid powder on the LD 50 of adrenaline shows it to be quite considerably reduced by thyroid powder, even to 1/7 or less. The reduction varies according to the dose, following quantitatively the increase in the amount of thyroid powder administered. In particular, if the dose employed is 1—9 mg/20 g, the change is dependent in detail on the dose, and a difference of 1 mg/20 g in the dose causes a distinctly perceivable difference in the LD 50. This means that differences in dosage of 30 per cent and even less can be clearly indicated in the variation of LD 50. Such high sensitivity, generally speaking, has not been present in the methods employed for the standardisation of thyroid powder.

The change in the lethal dose of adrenaline caused by thyroid powder is dependent on the time within which the powder has been administered, the effect appearing in about 4 days and reaching its maximum after 12 days. Subsequently, it remains the same, even after about 20 days, in spite of continued administering of the powder. That the reaction does not reach its maximum until after 12 days is naturally somewhat disadvantageous in standardisation, but the phenomenon is typical of thyroid powder, as its effect is slow of appearance. On the other hand, *e.g.* in the guinea-pig weight method, and many other reactions, the observation periods may even exceed 1 month. This indicates that a certain change takes place in the LD 50 with each dose of thyroid powder administered. The same observation can be made also of the changes in basal metabolism.

The changes, occasioned by thyroid powder, in the LD 50 of adrenaline are fairly little dependent on external factors. It has been possible to ascertain, for instance, that seasonal and nutritional variations occasion practically no changes, nor does the sex seem

to be of any importance in this respect. The changes in LD 50 occasioned by temperature and light correspond in the main to changes in the LD 50 of adrenaline without the effect of thyroid powder.

For the determination of one LD 50, test animals from a minimum of 25 up to 150 or more in number have been employed in the tests. The determination has been effected by administering at least two of each of the consecutive doses of the next size classification to the dose corresponding to LD 50 — larger and smaller — in the majority of cases even three or more. The size of the dose in both directions has, therefore, been confirmed by at least two doses, and a minimum of 5 doses has been employed to determine the LD 50. In each group the minimum of animals employed has been 5. If the accuracy of such a method is checked mathematically, it can be considered adequate for biological work. The dispersion present in test results is slight, the extent of probable error less than the variations obtained in the test results, and consequently the results must be considered to supply statistical evidence. The technical method of the tests is easy and requires no special equipment. The test animals employed are fairly cheap and easy to obtain, which enables their abundant use and increases the accuracy of standardisation. Hence, the reaction, from the technical point of view, can be considered well adapted for the determination of the biological activity of thyroid preparations.

SUMMARY

Numerous methods have been developed for the standardisation of thyroid preparations, based on different reactions occasioned by thyroidectomy on or administering of thyroid preparations to animals. Several methods are so little sensitive quantitatively that it has been possible to use them primarily for qualitative tests only. A few only — those based on increased basal metabolism, increased acetonitrile resistance, and reduction in weight induced by thyroid preparations — have also been employable in effecting quantitative determinations. A number of disadvantages, however, are inherent in the latter methods too, due to which they are either susceptible to several potential errors or technically difficult of execution. For this reason none of the methods has proved sufficiently reliable, and for practical use the determination must be effected simultaneously by several methods.

The object of the present investigation has been to study the suitability of a new phenomenon as a basis of a standardisation method — the reduction in the lethal dose of adrenaline occasioned by thyroid powder. To this end, investigations have been made into the lethal dose of adrenaline and the factors affecting it, into the reduction occasioned by thyroid powder in the lethal dose of adrenaline and the dependence of the said reaction on different factors. Of the factors possibly affecting the lethal dose of the adrenaline, the significance of the season, lighting, weight of animals, sex, nutrition, temperature and concentration of the adrenaline suspension injected, have been studied.

In the investigations into the reduction occasioned by thyroid powder in the lethal dose of adrenaline, on the other hand, the endeavour has been to throw light on the dependence of the change on the size of the thyroid powder dose, on the period of its administration, on the sex of the test animals, nutrition, lighting of the environment, temperature and season.

The test animals employed for the investigation totalled 1,452 mice, descended from three strains of mice and kept in as similar conditions as possible. The thyroid powder has been given to the animals in a water suspension injected into bread. The adrenaline used in the test has been administered by subcutaneous injections.

The following conclusions can be drawn from the test results:

LD 50 (the dose occasioning 50 per cent mortality) of adrenaline, with adult mice as test animals, is 7 γ/g. Variations in LD 50 may be produced by the temperature at which the test animals are kept — cold reducing the size of the lethal dose and heat having the opposite effect — and light — the lethal dose of adrenaline decreasing with animals kept in darkness, and the concentration of the adrenaline solution used — the lethal dose increasing if the concentration of adrenaline is diluted to 1 : 3 000 or more; between 1 : 1 000—1 : 2 000 no variation can be indicated. The lethal dose is independent of the weight of the test animals, provided adult mice are in question, of their sex, nutrition, and of the season.

Thyroid powder occasions a marked reduction in the LD 50 of adrenaline, the reduction being proportionate to the amount of thyroid powder. It is dependent on the period during which powder has been administered to the test animals, up to a certain limit, beyond which the effect ceases to increase in spite of the powder being administered continuously, provided the size of dose is kept constant. The effect is not dependent on the season of the year, weight, sex or nutrition, but is dependent to some extent on the lighting and temperature of the surroundings.

The dispersion found in studying the test results is so slight that the results must be considered as supplying statistical evidence.

The tests are technically easy of execution, and potential errors in the tests are easy to eliminate. Hence, the reaction studied in the present investigation can probably be considered well suited for use in the standardisation of thyroid preparations.

REFERENCES

Abderhalden, E. and Gellhorn, E.: Pflügers Arch. f. Physiol., 1923: 199:320.

Abelin, J.: Biochem. Zschr., 1923:137:273.

—»—: Pflügers Arch. f. Physiol., 1923:198:257.

—»—: Biochem. Zschr., 1928:149:72.

—»— and Sato, R.: Schweiz. med. Wschr., 1925:55:45.

—»— and Wegelin, C.: Klin. Wschr., 1932:11:2103.

Aehle, H.: Ibidem, 1935:14:638.

Asher, L.: Zschr. f. Biol., 1911:55:83.

—»— and v. Rodt, W.: Zentralbl. f. Physiol., 1912:26:223.

—»— and Streuli, H.: Biochem. Zschr., 1918:87:359.

Basinger, H.: Arch. Int. Med., 1916:17:260.

Belkin, B.: C. r. Acad. Sci. USSR, 1934:2:90.

Benedict, F.: Methoden des Gaswechsels, Abderhaldens Handbuch der biologischen Arbeitsmethoden, Wien, 1926:Abt.IV:T.10:413.

Blau, N.: J. Biol. Chem., 1935:110:351.

Büngeler, W.: Frankfurt. Zschr. f. Path., 1930:39:426.

Cameron, A. and Carmichael, J.: Trans. Roy. Soc. Canada, 1929: 23:169.

Chick, H. and Roscoe, M.: Biochem. J., 1930:24:1780.

Cramer, W.: Proc. Roy. Soc. London, 1913:86:550.

Csillag, E.: Pflügers Arch. f. Physiol., 1924:202:588.

de Quervain, F.: Schweiz. med. Wschr., 1923:53:10.

—»—: Ergebn. Physiol., 1925:24:701.

Deusch, M.: Deutsch. Arch. klin. Med., 1922:138:175.

Eiger, M.: Zschr. f. Biol., 1917:67:253.

Engländer, M.: Wien. klin. Wschr., 1925:7:193.

—»—: Ibidem, 1925:12:327.

Eppinger, H.: Pathologie und Therapie des menschlichen Ödems, Berlin, 1917.

Fischer, F. and Loew, W.: Med. Klin., 1934:30:200.

Foster, G.: J. Biol. Chem., 1929:83:345.

—»— and Sundström, E.: Ibidem, 1926:69:565.

Freud, P. and Nobel, E.: Klin. Wschr., 1924:3:1849.

Freund, H. and Gottlieb, R.: Arch. exper. Path., 1922:93:92.

Fukui, T.: Pflügers Arch. f. Physiol., 1925:210:410.

Fürth, O. and Schwarz, K.: Pflügers Arch. f. Physiol., 1908:124:113.

Gaddum, J.: J. Physiol., 1930:68:383.

—»—: Quart. J. Pharmacol., 1931:4:183.

Gardella, E.: Arch. di Fisiol., 1910:8:409.
Gellhorn, E.: Pflügers Arch. f. Physiol., 1923:200:571.
Glaubach, S. and Pick, E.:Arch. exper. Path., 1931:162:537.
—»— —»—: Ibidem, 1935:179:678.
Goetsch, E.: Long Island. Med. J., 1922:16:155.
Goldner, M.: Zschr. klin. Med., 1928:108:110.
Grab, W.: Arch. exper. Path., 1932:167:313.
Gudernatsch, J.: Amer. J. Anat., 1913:15:431.
—»—: Endocrinol., 1933:17:239.
Haffner, F.: Klin. Wschr., 1927:6:1932.
—»— and Komijama, T.: Arch. exper. Path., 1925:107:69.
Harington, C.: The thyroid gland, its chemistry and physiology, London 1933.
—»— and Barger, G.: Biochem. J., 1927:21:169.
—»— and Randall, S.: Ibidem, 1929:23:373.
Häusler, H.: Arch. exper. Path., 1936:181:345.
Hecht, G.: Ibidem, 1932:166:432.
Henning, H.: Zschr. f. d. ges. exper. Med., 1934:94:259.
Hesse, E., Jacobi, K. and Bregulla, G.: Arch. exper. Path., 1933: 170:13.
Hunt, R.: J. Biol. Chem., 1905:1:33.
—»—: J. Pharmacol., 1910:2:15.
—»—: Amer. J. Physiol., 1923:63:257.
—»—: Arch. Int. Med., 1925:35:671.
Jorpes, E. and Wilander, O.: Nordiska Specialitets Komissionens Aarsberettelse, 1933.
Juhn, M. and Barnes, B.: Amer. J. Physiol., 1931:98:463.
Kanitz, H. and Apitzsch, J.: Biochem. Zschr., 1934:272:189.
Kendall, E.: J. Biol. Chem., 1919:39:125.
—»—: Ibidem, 1928:80:357.
Kocher, A.: Mitt. Grenzgeb. Med. u. Chir., 1917:29:309.
Kraus, F.: Berl. klin. Wschr., 1908:45:1709.
Kreitmair, H.: Zschr. f. d. ges. exper. Med., 1928:61:202.
—»—: Ergebn. Physiol., 1930:30:202.
Krogh, M. and Lindberg, A.: Bibliot. f. Laeg., 1931:123:362.
Küchler, W.: J. Ornithol., 1935:83:414.
Kuschinsky, G.: Arch. exper. Path., 1935:179:726.
Leland, J. and Foster, G.: J. Biol. Chem., 1932:95:165.
Loeser, A. and Eitel, H.: Arch. exper. Path., 1932:167:381.
Marti, H.: Zschr. f. Biol., 1923:77:181.
Meyer, F.: Biochem. Zschr., 1929:208:127.
Miura, M.: J. Labor. a. Clin. Med., 1922:7:349.
Mörch, K.: Dansk Tidskr. f. Farm., 1928:281.
Nordiska Specialitetskomissionens Årsberättelse, 1932.
Oberdisse, K.: Arch. exper. Path., 1931:162:150.
Oswald, A.: Pflügers Arch. f. Physiol., 1916:164:506.
Paal, H.: Klin. Wschr., 1933:12:1988.

Paladino, R.: Biochem.Zschr., 1912:42:302.
Parhon, M.: J. Physiol. et Path. gén., 1913:15:75.
Paus, B.: Nord. Med., 1947:34:867.
Peltola, P.: To be published.
Peltola, P. and Vartiainen, A.: Acta Soc. Med. Fenn. Duodecim, 1945:24:91.
Pelyi, J.: Biochem. Zschr., 1935:276:376.
Pick, E. and Pineles, F.: Zschr. exper. Path. u. Ther., 1910:7:518.
Reiss, M.: Endocrinol., 1932:11:97.
Romeis, B.: Arch. Entw. Mechan., 1913:37:183.
Rotter, G.: Arch. exper. Path., 1932:166:649.
Salter, W. and Lerman, J.: J. Clin. Invest., 1935:14:691.
Santo, E.: Zschr. f. d. ges. exper. Med., 1934:93:793.
Smith, P.: Amer. J. Pathol., 1927:3:669.
Solé, A.: Zschr. f. d. ges. exper. Med., 1928:59:45.
Storm van Leuwen, W. and van den Made, M.: Arch. exper. Path., 1920:88:318.
Takahashi, Y.: Biochem. Zschr., 1924:145:130.
Todd, T. and Wharton, R.: Amer. J. Anat., 1934:55:97.
Trendelenburg, P.: Biochem. Zschr., 1910:29:396.
—»— and Vogt, R.: Quoted by Bomskow: Methodik der Hormonforschung, Leipzig, 1937.
Uhlenhuth, E.: Endocrinol., 1922:6:102.
—«—: Arch. Entw. Mechan., 1929:119:516.
Wahlberg, J.: Studien über die Schilddrüsenkrankheiten in Finnland, Helsinki, 1938.
Vartiainen, A.: Personal communication, 1950.
Woitkewitsch, A.: Virchows Arch. f. path. Anat., 1935:294:653.
Zawadowsky, B.: Endocrinol., 1932:10:23.